THE "TOXIN" IN CLOSED-LOOP STRANGULATION OBSTRUCTION*

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Two papers which appeared about 1915 are the basis for almost all work that has since appeared in the field of closed-loop strangulation obstruction. The papers by Murphy and Brooks,[1] and by Dragstedt, Moorhead, and Burcky[2] contained a tremendous amount of information which was far in advance of their times, and the work of these two groups laid the groundwork for almost everything that has appeared subsequently. Either by actual experimentation, or by prediction, they covered almost every conceivable phase of the closed-loop problem. Since then, additions based upon new techniques or new drugs not available to these workers have only built upon ideas which were predicted in their early work.

The literature on strangulation obstruction is too extensive to be reviewed at any one time, and even that portion of the literature devoted to the study of toxicity of the closed loop has become so extensive as to warrant very careful reading to be adequately evaluated. Since this literature has been recently reviewed,[3] it seemed preferable to summarize some ideas from our own experimental laboratory, as well as some ideas from other laboratories.

Our own work[4] with the closed loop began as a result of our failure to achieve the expected results in the study of strangulation obstruction in a Thiry fistula. When animals with a strangulated Thiry fistula did not routinely succumb even after the Thiry fistula was irrigated with large quantities of pure bacterial cultures, it became apparent that some important factor was missing. Eventually, it became clear that the missing factor was distention, which does not occur in a Thiry fistula,

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since it continually drains to the outside and decompresses itself. In an attempt to overcome this difficulty, studies were conducted with a Thiry fistula which was occluded by a balloon inserted in the open draining end. In order to produce distention of the Thiry fistula, however, this balloon had to be distended to such a degree that it caused necrosis of the adjacent bowel wall and this study had to be abandoned. It then occurred to us that the problem could be adequately studied by using an isolated closed loop of small bowel, with intestinal continuity being restored by making an intestinal anastomosis around the closed loop. Such a closed loop had been used repeatedly in the past, but purposeful strangulation of such a loop had not met with survival of the experimental animal. It therefore seemed that strangulation of a closed loop of small intestine should put any form of therapy to an extreme test.

Accordingly, a 15-cm. loop was selected from the distal small bowel of dogs and the two ends of the loop were closed after the loop had been irrigated with sterile saline.[4, 5] An end-to-end intestinal anastomosis was made around the loop. A plastic tube was threaded into the closed loop, so that antibiotics could be placed directly into the lumen of the closed strangulated loop of bowel in the postoperative period. The treated animals received antibiotics into the closed loop for five days following strangulation. The control animals received no antibiotic therapy of any kind. All animals were given fluid support during the first 24 to 48 hours after strangulation. The omentum was removed in all animals, so that it could not provide additional vascular support to the strangulated small bowel.

Ten of eleven control animals died within 144 hours after strangulation, with an average survival time of 77 hours. One animal survived to be re-explored at 144 hours, but this animal's survival has not been explained. In contrast to the relatively short survival periods of the control animals, all but two of the treated animals lived beyond 144 hours, some of them surviving six months before the closed loop was resected for further study. When the animals were re-explored from one week to six months later, it was noted that the previously strangulated loop had returned to a grossly normal appearance, that new vessels had developed in the mesentery of the small bowel, that other loops of bowel had become adherent to the strangulated loop, and that new blood vessels accompanied the adhesions to other loops of

bowel. In the animals that survived the longest, the serosal surface had its normal sheen, the bowel was grossly and histologically normal, and some of the closed loops had distended to three to five times the size of the normal small bowel.

Cultures taken from the lumen of the closed loop prior to its irrigation showed no difference in bacterial flora between the control and the treated animals. Cultures taken after irrigation with the antibiotic solution in the treated group showed that this technique had served essentially to sterilize the loop. Cultures taken at the time of re-exploration in the treated animals showed the continued absence of a bacterial flora. These studies have been confirmed by Allcock in a report which arrives at almost identical conclusions throughout.[6] Thus, it was shown that intraluminal antibiotics would protect a closed strangulated loop of bowel even in the presence of the tremendous distention which can occur in a closed loop. The importance of bacteria and the protective effect of antibiotics under the extreme stress of a strangulated closed loop of bowel was once again demonstrated.

Barnett has reported that the peritoneal fluid from dogs with a closed-loop strangulation obstruction was toxic when injected intraperitoneally into normal dogs, and that this toxicity was mainly the result of bacteria present in the peritoneal fluid.[7-14] Our experience and Barnett's own articles seemed to indicate that the majority of experiments on which such a conclusion could be based were those in which the closed loop had perforated. Therefore, he was studying a type of fecal or bacterial peritonitis, rather than the toxicity of strangulation obstruction *per se*. Since our experience did not indicate that bacteria in the peritoneal fluid were the cause of death in strangulation obstruction, it was decided to repeat Barnett's experiments. Barnett reported that this fluid was toxic when injected intraperitoneally into normal animals in a dosage of 3 ml./kg. of body weight. He also reported that the late peritoneal fluid from strangulated animals caused such a painful reaction that recipient dogs had to be anesthetized. Since this was also at variance with our experience, we repeated the study, injecting the fluid into both anesthetized and nonanesthetized animals.[15] When the terminal peritoneal fluid from dogs with a closed-loop strangulation obstruction was injected intraperitoneally into anesthetized test dogs in a dosage of 3 ml./kg. of body weight, there was a 100 per cent mortality. On the other hand, if the test animals

were not anesthetized it was possible to inject 3, 6, or 9 ml./kg. without a lethal result. It would seem, therefore, that bacteria in this peritoneal fluid are not the sole cause of death and that Barnett's conclusions should be carefully restudied. Toxicity of the peritoneal fluid should be tested uniformly in anesthetized animals or in nonanesthetized animals. The specific role of anesthesia in enhancing the toxicity of this peritoneal fluid is not understood, and it is hoped that this problem will be investigated more fully. More recently, Barnett[16] has suggested that the toxicity in closed-loop strangulation obstruction is the result of bacterial cells and bacterial endotoxins, and that studies with specific anti-endotoxin agents suggest these are of some value in treatment.

Laufman and Nora[17,18] reported experiments in which it was shown that bacteria could proliferate in the veins draining a strangulated segment of bowel. They suggest that release of these organisms and their toxins into the systemic circulation at the time of resection or release of the strangulating mechanism would permit onset of all the late symptoms of strangulation obstruction. Cultures from veins draining a strangulated segment of bowel lend support to this hypothesis.

Because there are certain advantages of the study of a problem in smaller animals where larger numbers of experiments can be done and where the operations can be carried out with greater facility, it was decided to restudy the problem of closed-loop strangulation obstruction in rabbits.[19] Furthermore, the intestinal bacteriology of the rabbit is quite different from that of dog or man, and it was thought that this different native intestinal flora might yield some difference in results which might help to explain certain facets of the problem.

Peritoneal fluid has been collected from 54 rabbits with a closed-loop strangulation obstruction. This peritoneal fluid has been pooled and tested for toxicity in mice, since the use of a small animal allows for a larger number of test experiments and repetitive studies on the same sample. The validity of testing the toxicity of fluid from one animal species in another had previously been established by the demonstration that, on a comparable dosage schedule, the results obtained in dogs and in mice were comparable. The pooled peritoneal fluid from rabbits was subjected to high-speed centrifugation, sterilized by filtration, and then tested for both toxicity and sterility.

When 0.1 ml. sterile supernate obtained in this fashion was injected

intraperitoneally into mice, 14 of 17 mice succumbed within 24 hours after injection. If the mice were pretreated with 0.2 ml. of normal horse serum, 19 of 19 animals died, showing that this had no protective effect. On the other hand, if the mice were pretreated with 0.2 ml. of pentavalent gas gangrene antitoxin, only one of 19 died, thus demonstrating that neutralization of clostridial toxins with pentavalent gas gangrene antitoxin protected the mice from the lethal effects of the strangulation obstruction peritoneal fluid.

In studies with each of the five individual clostridial antitoxins it was found that none of the animals died when pretreated with either *Clostridium novii* antitoxin or *Clostridium sordelli* antitoxin. In contrast, when the animals were pretreated with *Clostridium perfringens*, *Clostridium septicum*, or *Clostridium histolyticum* antitoxins, all were dead within 48 hours. Subsequent work has shown that the antitoxin for *Clostridium sordelli* is the only one responsible for these protective effects. Comparable studies have been conducted in rats, guinea pigs, dogs, and also with the peritoneal fluid from a few patients with strangulation obstruction. The toxin of *Clostridium sordelli* has been demonstrated to be the major preformed lethal factor in closed-loop strangulation obstruction in rabbits and dogs. These studies indicate that clostridial exotoxins are a very important factor in the over-all toxicity of strangulation obstruction.

Since all of these studies have implicated clostridial exotoxins, it seemed that an ideal way to redirect our attention to the bacterial aspect of strangulation obstruction would be in the germ-free animal where bacteria and bacterial products would be totally excluded and where even longer survival might be anticipated, if bacteria are truly the cause of death in these animals. Accordingly, it was planned to conduct some studies in germ-free guinea pigs. However, in order to obtain the necessary base-line information, a series of studies of strangulation obstruction were conducted on conventional guinea pigs.[20] Since the guinea pig is another small animal which can be studied in large numbers in a short time, studies were carried out using a closed loop in guinea pigs that received no treatment; and using an open loop in guinea pigs that were divided into four groups, one of which received no therapy, one of which received antibiotics alone, one of which received fluids alone, and one of which received both antibiotics and fluids. Closed-loop strangulation obstruction was again shown

to be the most severe type of strangulation obstruction and one of the most difficult to treat. The poor survival in the closed-loop group re-emphasizes the importance of the survival obtained in the dogs with antibiotics in the lumen of the closed loop.

With this as a prelude, germ-free studies were initiated, but unfortunately we were not successful in raising germ-free guinea pigs. We subsequently switched our attention to germ-free dogs and have been successful in studying strangulation obstruction in germ-free pups.[21,22] Our original experiences with animals in germ-free isolators provided much experience with the problems and difficulties in the use of germ-free animals for major surgical procedures. However, some of these problems have subsequently been resolved, and it has been possible to study strangulation obstruction in germ-free pups, in limited flora pups, and in conventional pups of the same age. Three germ-free pups between 14 and 24 weeks of age have survived a strangulation obstruction for 100 hours, after which the strangulated segment of bowel was resected and intestinal continuity restored, with survival of the animals. The strangulated segment was revascularized and was grossly and histologically viable. Five additional limited-flora pups contaminated with only staphylococci and bacilli also survived to be re-explored at the same time. Animals contaminated with clostridia survived for approximately the same time as did the conventional pups, and all had grossly gangrenous bowels at autopsy. Somewhat similar results were obtained with germ-free rats, but difficulties with anesthesia complicated these experiments. Amundsen and Gustafsson[23] have reported prolonged survival in strangulated germ-free rats, using a slightly different technique. The improved survival with germ-free animals emphasizes once more the importance of bacteria in strangulation obstruction.

Since all of this work has indicated the importance of bacteria and of bacterial products, it is of obvious importance to study the emergence of the bacterial flora within the strangulated loop of bowel. Most studies of intestinal bacteriology in strangulation obstruction have been limited to a study of the intestinal flora, either at the time of strangulation and/or at the time of autopsy. Serial studies of the change in the bacterial population have not been reported previously and, therefore, it seemed worthwhile to plan to sacrifice a series of animals at different intervals following strangulation for the specific

purpose of doing qualitative and quantitative bacteriologic studies at each of these time intervals. [24-26]

The bacterial population increases much more rapidly than had been anticipated, and the total bacterial population reaches almost a maximum within approximately six hours after strangulation, with a tremendous increase in numbers of clostridia, streptococci, and coliforms within this brief period of time. The very fact that these organisms can increase at this tremendously rapid rate means that exotoxins may be produced much more rapidly and in much larger quantities than had been previously appreciated. If these exotoxins can be absorbed by any route, their effects may be noted at an earlier period than had previously been realized. Thus, another type of study has again emphasized the importance of the intestinal bacterial flora in strangulation obstruction.

All recent studies from this and other laboratories have pointed more and more to a prominent role for bacteria in the cause of death in otherwise properly treated strangulation obstruction. The major arguments between various laboratories at the present time seem to depend upon whether the toxicity lies with the bacteria, with bacterial endotoxins, or with bacterial exotoxins. Work from our own laboratory lends support to the conclusion that bacterial exotoxins are the major factor in the cause of death in strangulation obstruction. It is hoped that further studies may more precisely identify the specific bacterial toxins involved, so that an attempt may be made to combat this toxicity with an antitoxin and, hopefully, thus improve clinical results in patients with strangulation obstruction.

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